

Operon concept

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A decorative graphic consisting of several parallel white lines of varying lengths, slanted diagonally from the bottom right towards the top right, located in the lower right quadrant of the slide.

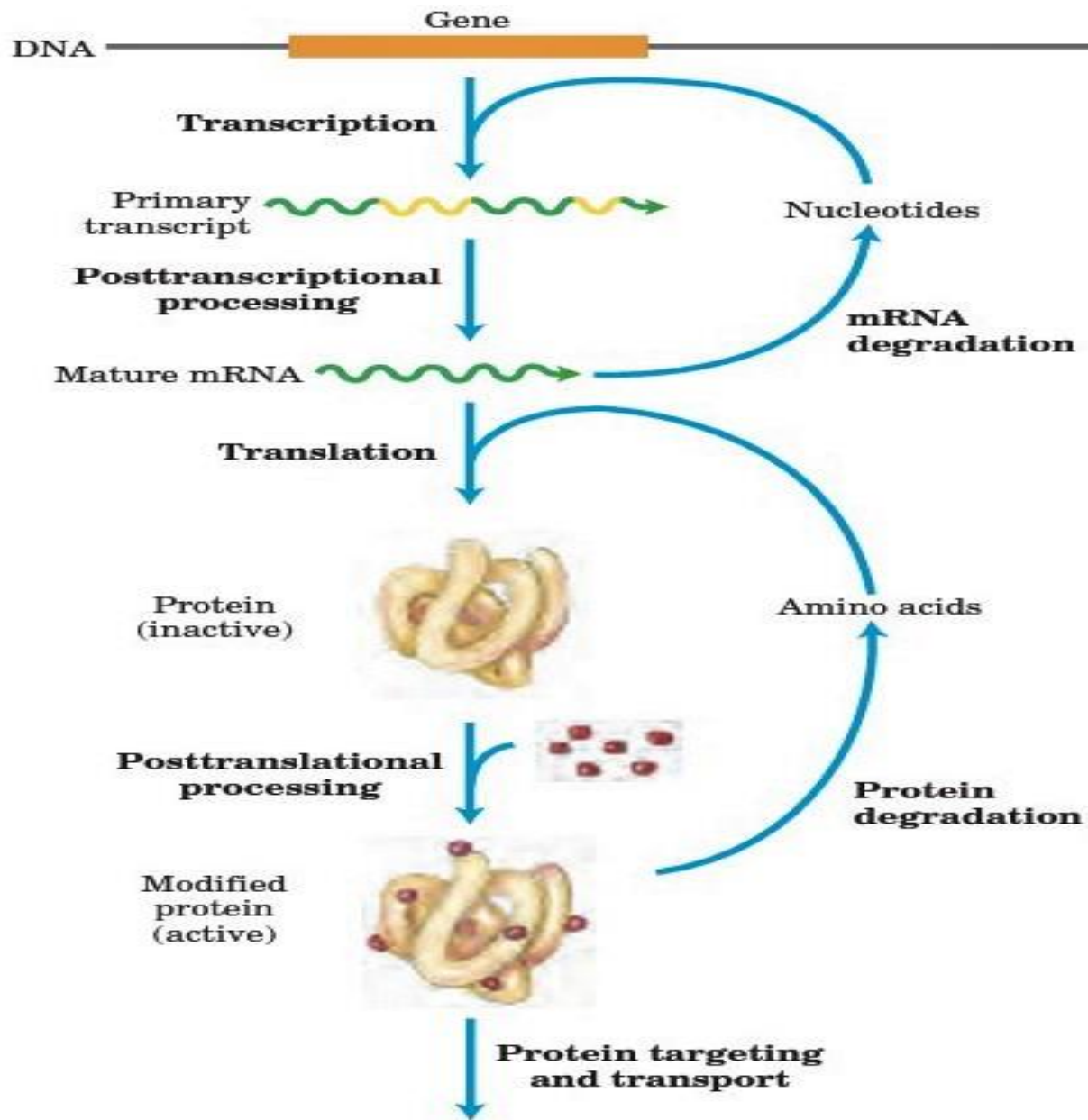


FIGURE 28-1 Seven processes that affect the steady-state concentration of a protein. Each process has several potential points of regulation.

What do you mean by operon

An operon is a genetic unit wherein a number of genes are under coordinate regulation by a single promoter.

An operon has several components:

1st, **structural genes** that encode the enzymes/proteins.

2nd, **regulatory genes** that encode regulatory proteins (trans-acting molecules), which either promote or reduce structural gene expression.

3rd, **regulatory sites** (cis-acting elements) where regulatory proteins bind and control gene expression.

What types of operons are there?

Depending on type of regulatory proteins, operon are of two types:

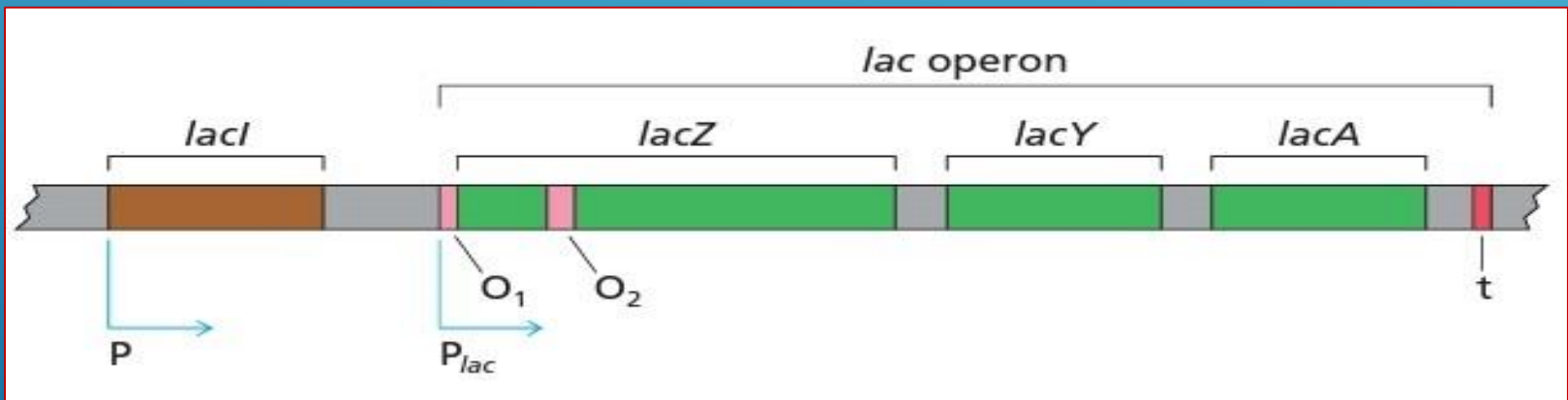
one is **inducible operon** where regulatory protein termed repressor (active) blocks gene expression. However, when effector molecule termed as **inducer** present, it blocks repressor activity, thus **stimulating gene expression**.

Another is **repressible operon** where regulatory protein termed as repressor (inactive) cannot blocks gene expression. However, when effector molecule termed as **co-repressor** present, it combines with inactive repressor to form active repressor which inturns **block gene expression**.

Thus, in inducible operon, transcription is normally off, whereas in repressible operon, transcription is normally on.

Lac operon

Lac operon is an example of inducible operon that has two independent genetic systems. One is promoter-operator-lacZ-lacY-lacA system and another is promoter-lacI system.



Components of lac operon

	<i>p</i>	<i>lacI</i>	<i>p_{lac}^O</i>	<i>lacZ</i>	<i>lacY</i>	<i>lacA</i>
DNA						
bp		1080	82	3069	1251	609
mRNA						
Polypeptide	Amino acids	360		1023	417	203
	kD	38.6		116.4	46.5	22.7
Protein	Structure	Tetramer		Tetramer	Membrane protein	Dimer
	kD	154.4		465	46.5	45.4
Function		Repressor		β -Galactosidase	Permease	Trans-acetylase

Functional attributes of lac operon components

lacZ encode **beta-galactosidase** enzyme (tetramer) that hydrolyzes lactose into glucose and galactose. It also catalyzes isomerization of lactose to allolactose that acts as inducer.

lacY encode **galactoside permease/M-protein** (monomer) that transport lactose into the cell.

lacA encode **thiogalactoside transacetylase** enzyme (dimer) that transfers an acetyl group from acetyl-coA to beta-galactoside. This enzyme is not essential for lactose metabolism. It appears to play a role in the detoxification of thiogalactosides that are also imported by the permease.

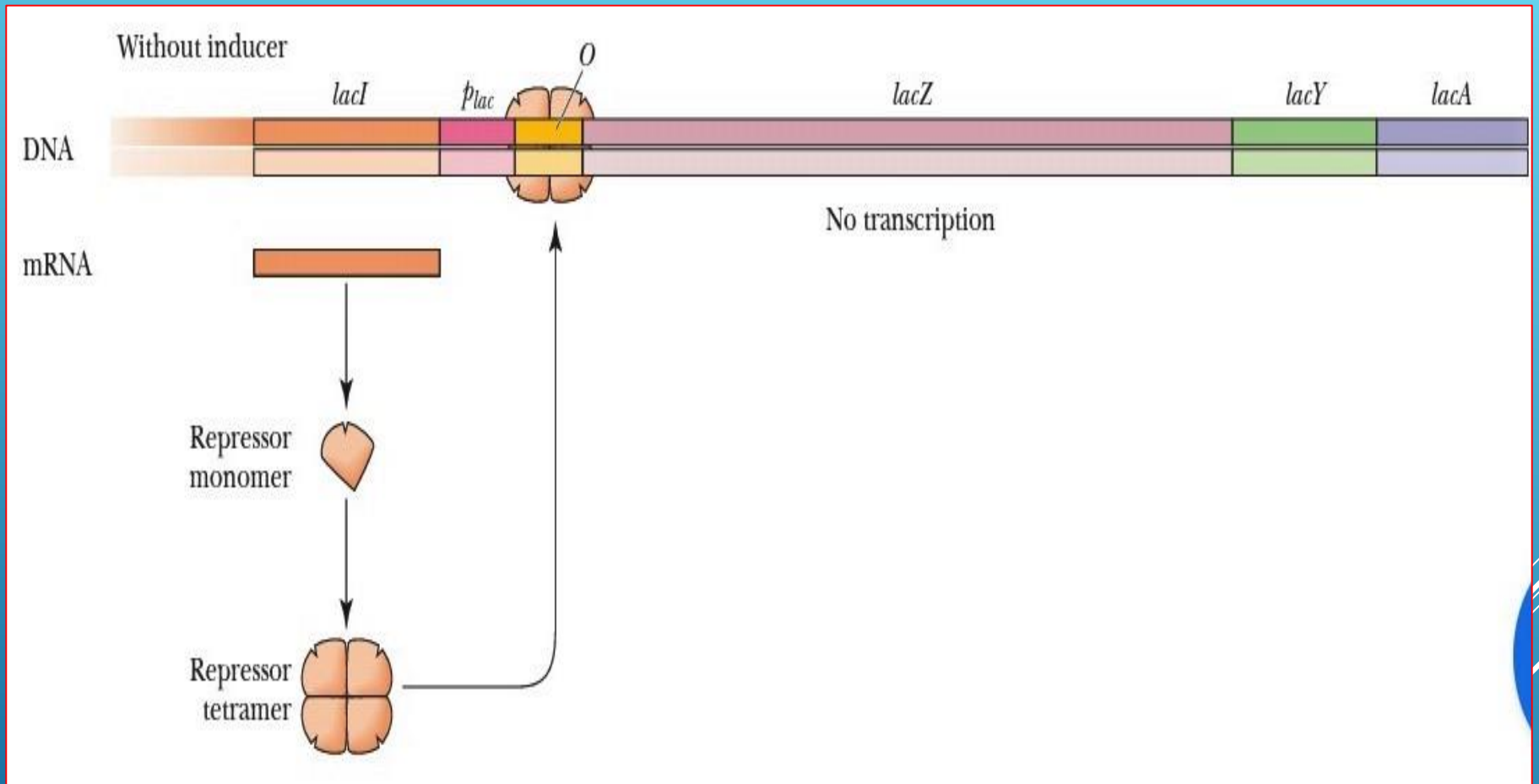
lacI encode regulatory protein called **repressor** that binds with operator site and interferes with binding of RNA polymerase with promoter or formation of open complex.

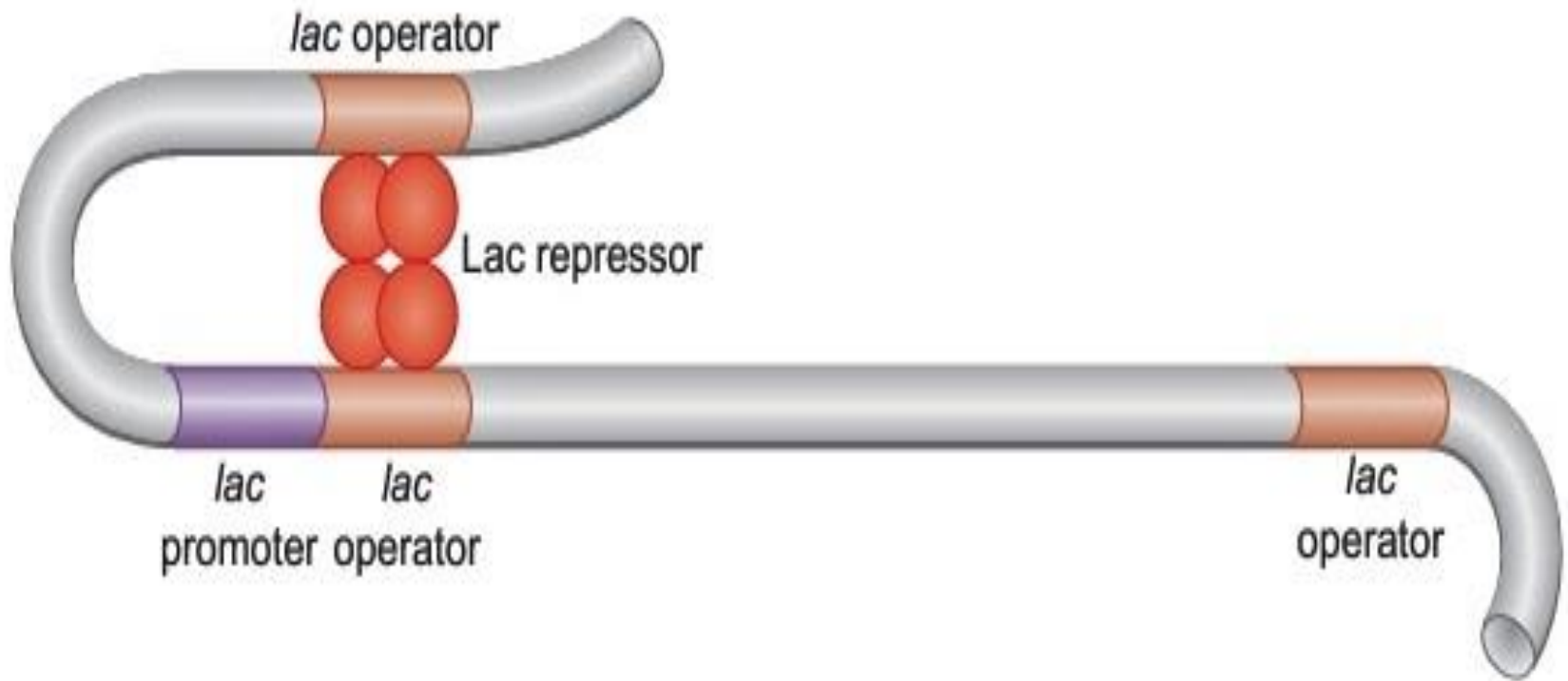
Promoter is a region of DNA/gene where RNA **polymerase binds** and initiates transcription.

Operator is a region of DNA/gene where **repressor protein** (product of regulatory gene lacI) binds and regulates transcription of structural genes of lac operon. Lac operon has three operator sites: one primary/major operator O₁ and two secondary operators O₂ and O₃.

Regulation of lac operon in the absence of lactose

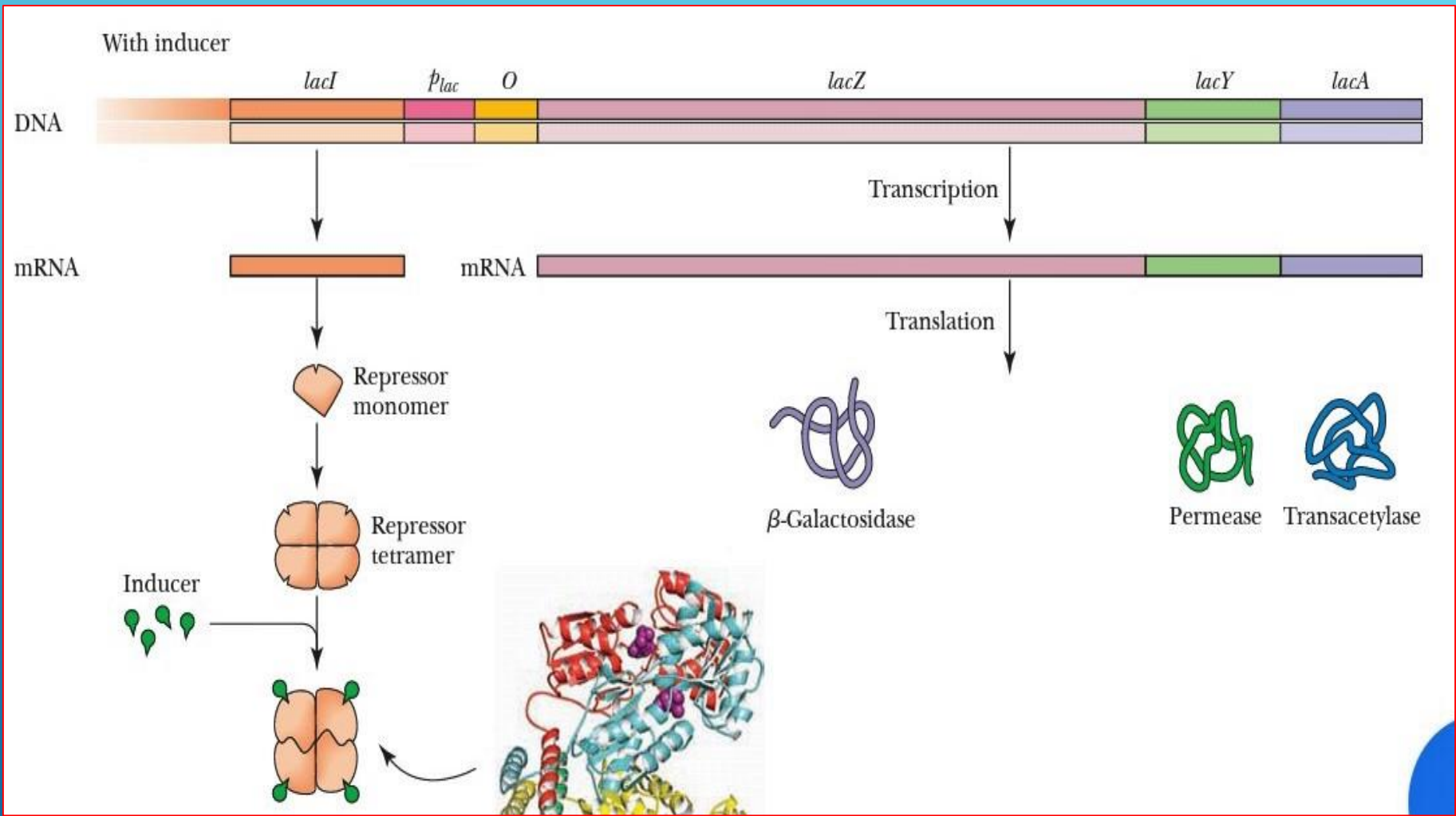
In the absence of lactose (inducer), free repressor tetramer binds to the operator DNA and so sterically prevents the binding of RNA polymerase to the promoter region. As a result transcription of three genes cannot be initiated.....because of this the lac operon is under the negative control. But, repressor does not bind to the operator permanently, i.e., it binds and dissociates. Thus, RNA polymerase can bind to the promoter and initiates transcription in between the time when one repressor dissociates and another repressor binds. This leaky expression produces few molecules of three enzymes that are essential for entry of lactose into the cell and conversion of lactose to allolactose (actual inducer) when lactose is first added.





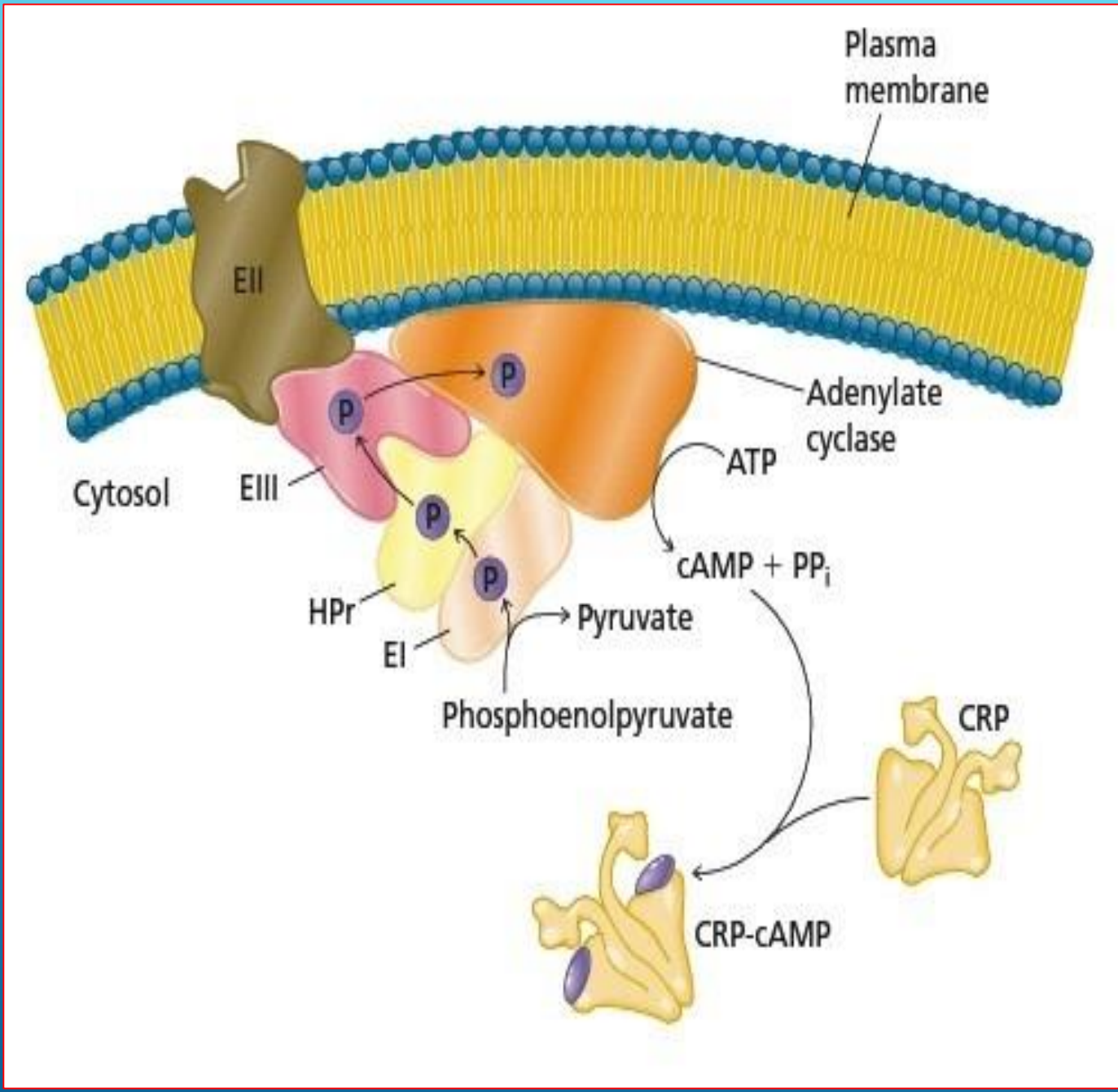
Regulation of the lac operon in the presence of lactose

In the presence of lactose, beta-galactosidase enzyme converts some molecules of lactose into allolactose. When this allolactose binds to repressor, it causes a change in the conformation (shape) of the repressor. As a result, repressor loses its affinity for the operator and dissociates from it. Now RNA polymerase can bind to the promoter and initiates transcription of three genes.



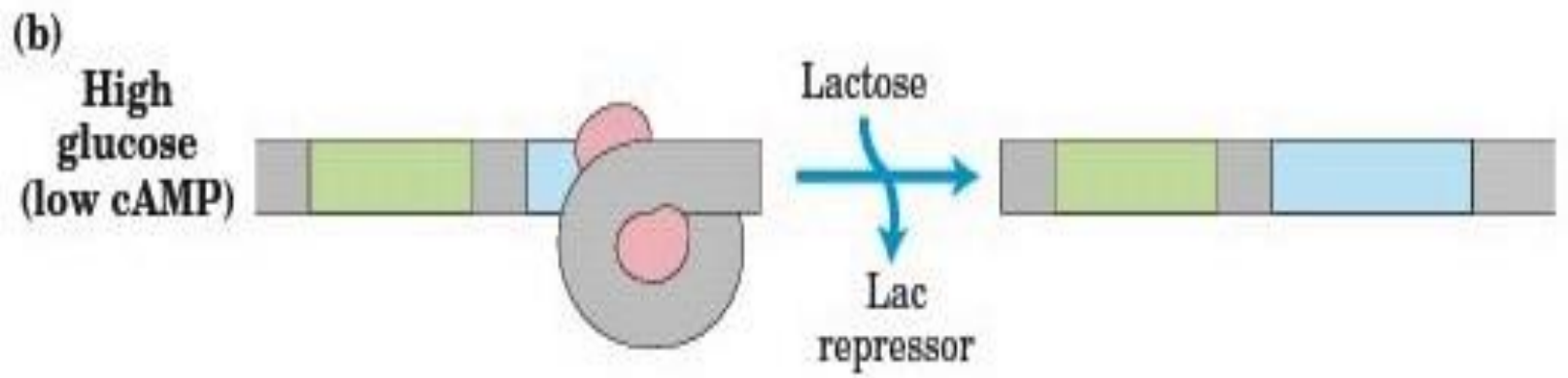
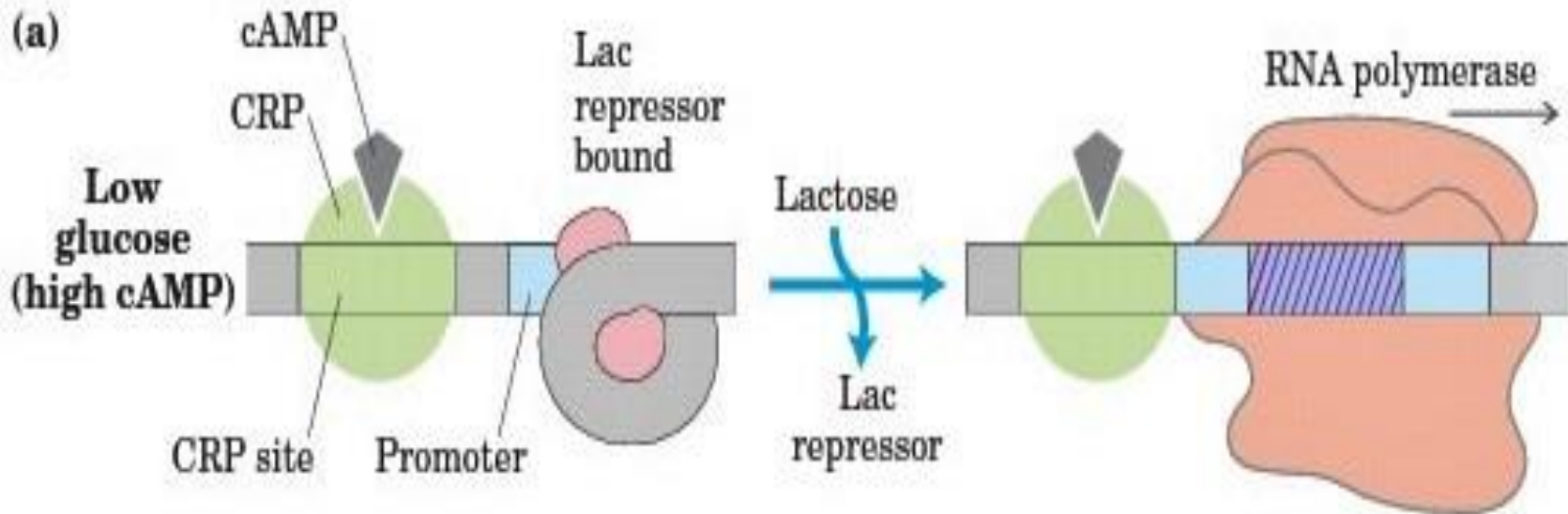
Regulation of lac operon in the absence of glucose

In the absence of glucose but the presence of lactose, the concentration of cAMP is high in a cell. This increased level of cAMP then form a complex with catabolite activator protein (CAP)/cAMP receptor protein (CRP). The resulting cAMP-CAP complex then binds to the CAP site (located just upstream of -35 site of the promoter) and increases the affinity of RNA polymerase for the promoter, thus enhancing transcription ~ 50 fold.



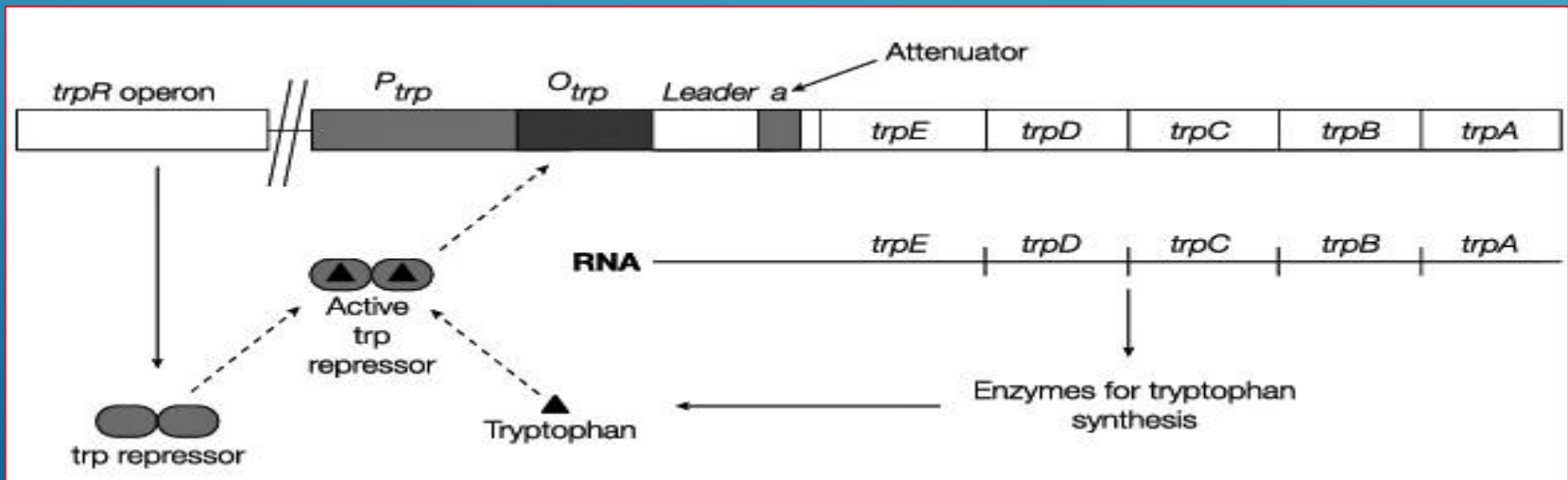
Regulation of lac operon in the presence of glucose

In the presence of both glucose and lactose, lac operon expressed at low level. The presence of glucose lowers the concentration of cAMP in the cell. As a result, the level of cAMP-CAP complex becomes lower which inturns reduces the binding of this complex for the CAP site, even though repressor is removed from the operator by the presence of lactose/allolactose. Thus, RNA polymerase cannot bind effectively to the promoter due to insufficient level of cAMP-CAP complex. Hence, the rate of transcription is decreased under this circumstance. During the process of initiation of transcription, the CAP contacts directly with RNA polymerase which was observed by photo-crosslinking.



Trp operon

Tryptophan operon is a repressible operon that has two independent genetic systems: one is promoter-operator-*trpE*-*trpD*-*trpC*-*trpB*-*trpA* system and another is promoter-*trpR* system. In the former system, there is a leader region termed as *trpL* in between operator and *trpE* gene.



Functional attributes of trp operon components

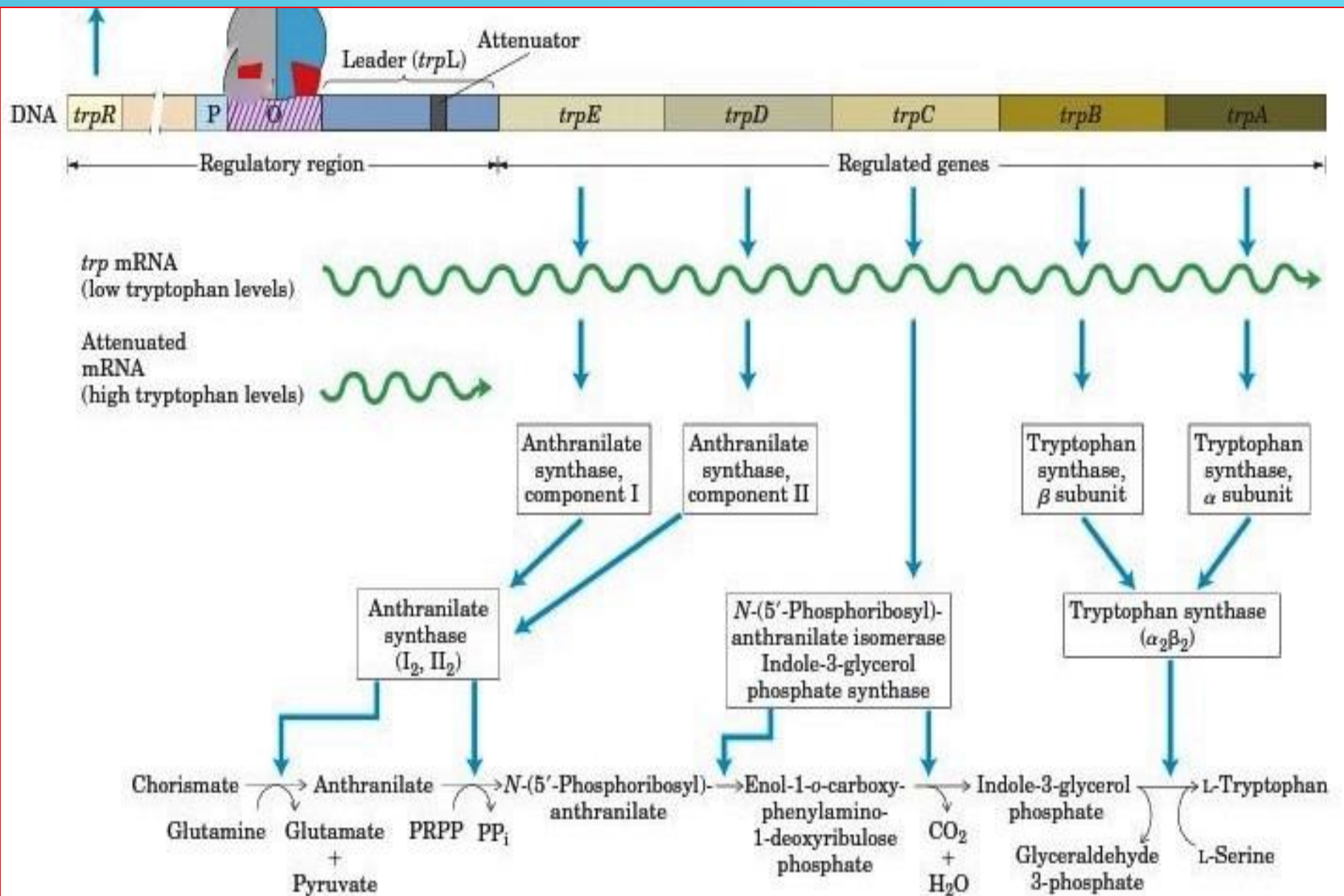
The **five structural genes (trpE through trpA)** encode the five polypeptides that assemble into **three enzyme complexes** catalyzing tryptophan synthesis from chorismate.

The **regulatory gene trpR** encodes an **aporepressor** protein which is a homodimer of two identical subunits, each with 107 amino acid residues.

Tryptophan operon has **two promoter sites**: one primary promoter P1 (40 nt) located upstream of trpE gene and another secondary weak promoter P2 located at distal end of trpD gene between trpD and trpC genes.

Tryptophan operator is a 21-bp sequence that is located within the primary promoter. As the operator and promoter completely overlap, binding of repressor to the operator DNA blocks the RNA polymerase's access to the promoter, thereby inhibiting gene expression.

A second regulatory sequence termed as **trp leader** is located between the operator and trpE gene. This trpL encodes a 162-nt long mRNA called **leader transcript** that has four regulatory regions (1 through 4) that can fold and form secondary stem-loop or hair-pin structures by complementary base pairing.



Mechanisms of trp operon regulation

The expression of the tryptophan operon is regulated by two different mechanisms:

- 1st mechanism is called **repression** that inhibits the initiation of transcription,
- 2nd mechanism is called **attenuation** that interfere elongation of transcription (premature transcription).

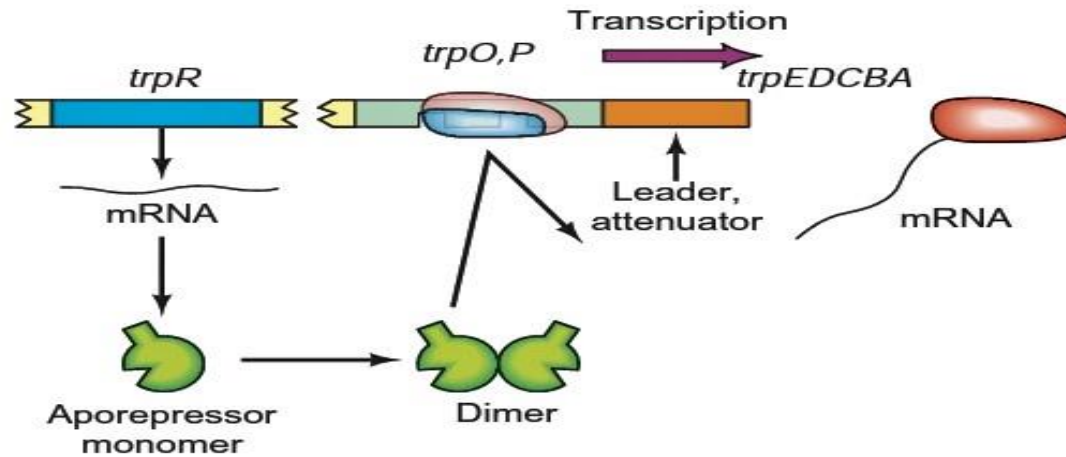
Regulation of trp operon in the absence of tryptophan

In the absence of tryptophan, the dimeric repressor is inactive that cannot bind to the operator DNA of Trp-operon and so RNA polymerase binds to the promoter and activates transcription of five structural genes for tryptophan biosynthesis (derepression).

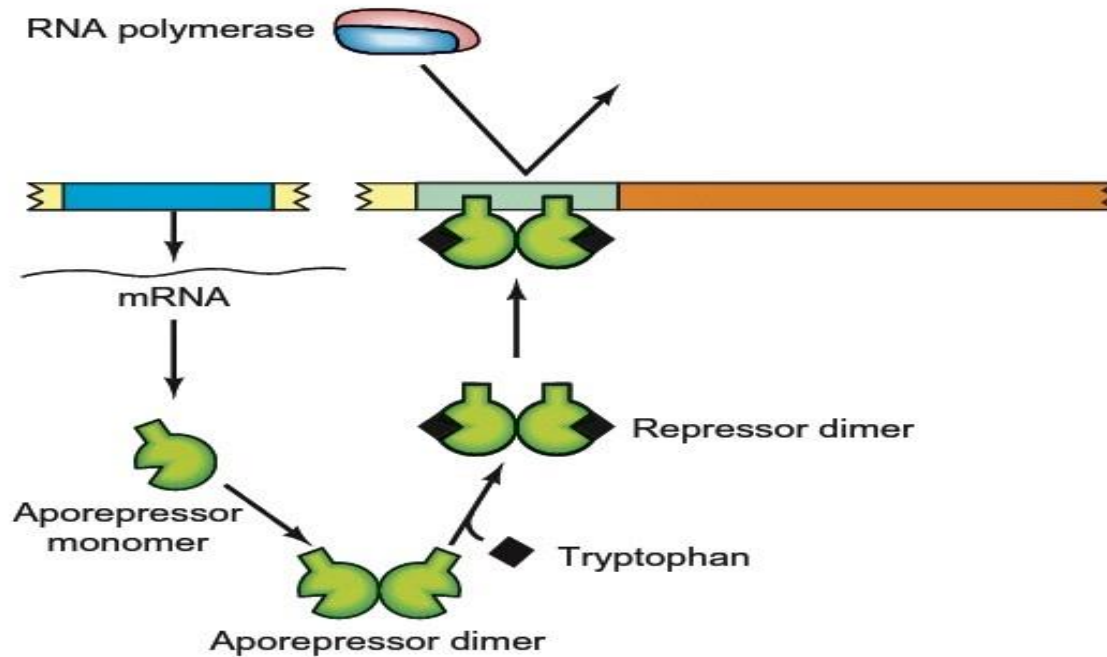
Regulation of trp operon in the presence of tryptophan

However, when tryptophan is present, it acts as co-repressor and binds with inactive apo-repressor to form active holo-repressor complex. The tryptophan activates apo-repressor by inducing a conformational change or allosteric shift in the inactive repressor. The active repressor complex then binds to the operator DNA of the operon and blocks transcription initiation by interfering with binding of RNA polymerase to the promoter.

(a) Low tryptophan: no repression



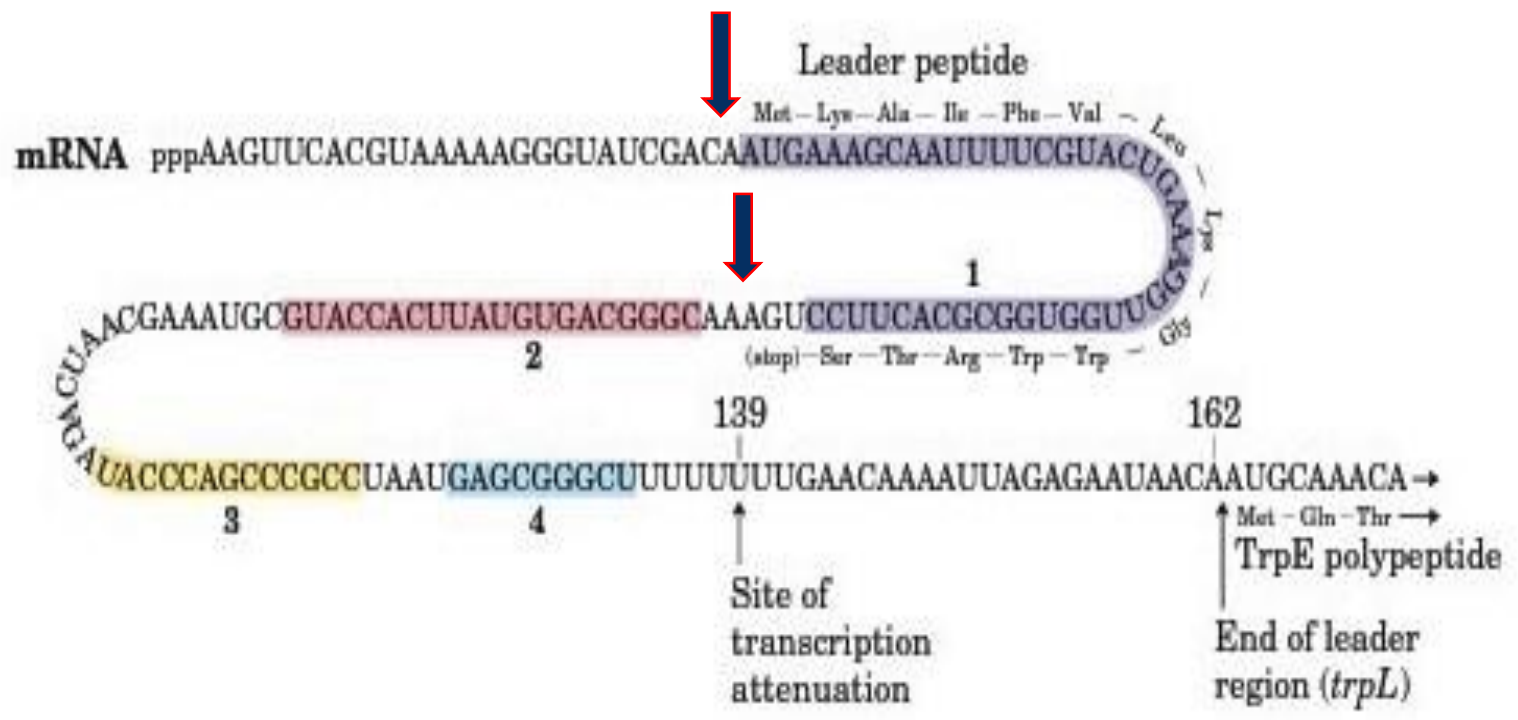
(b) High tryptophan: repression

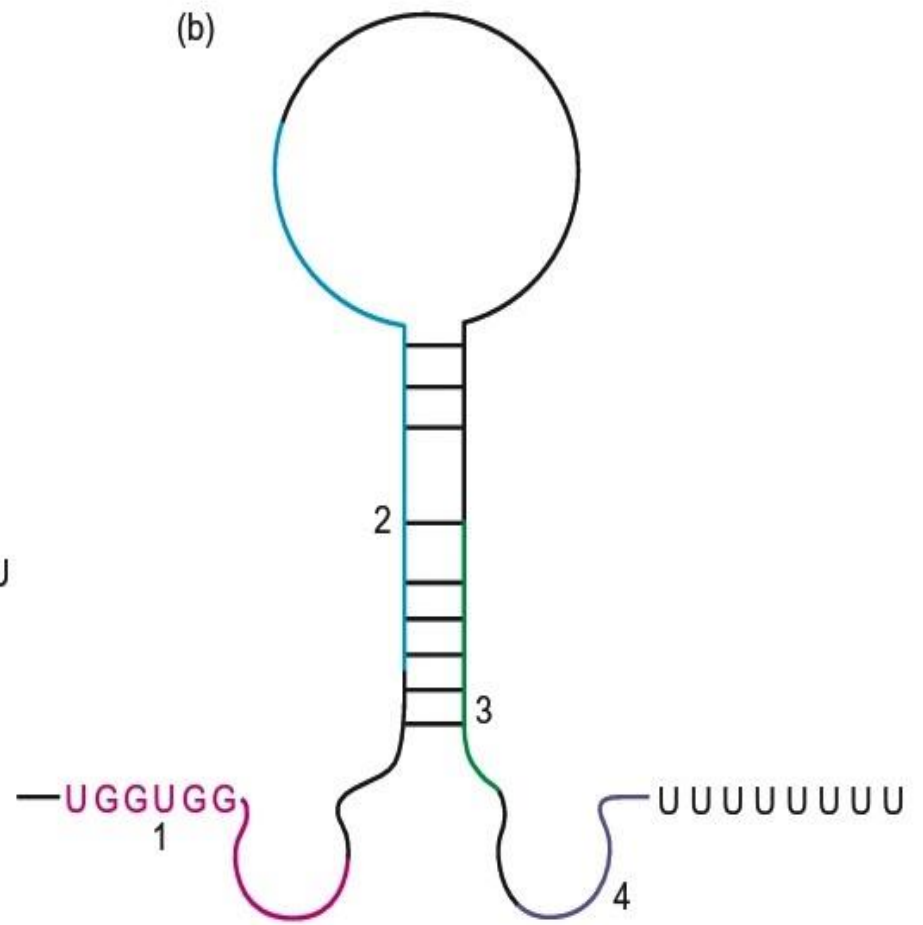
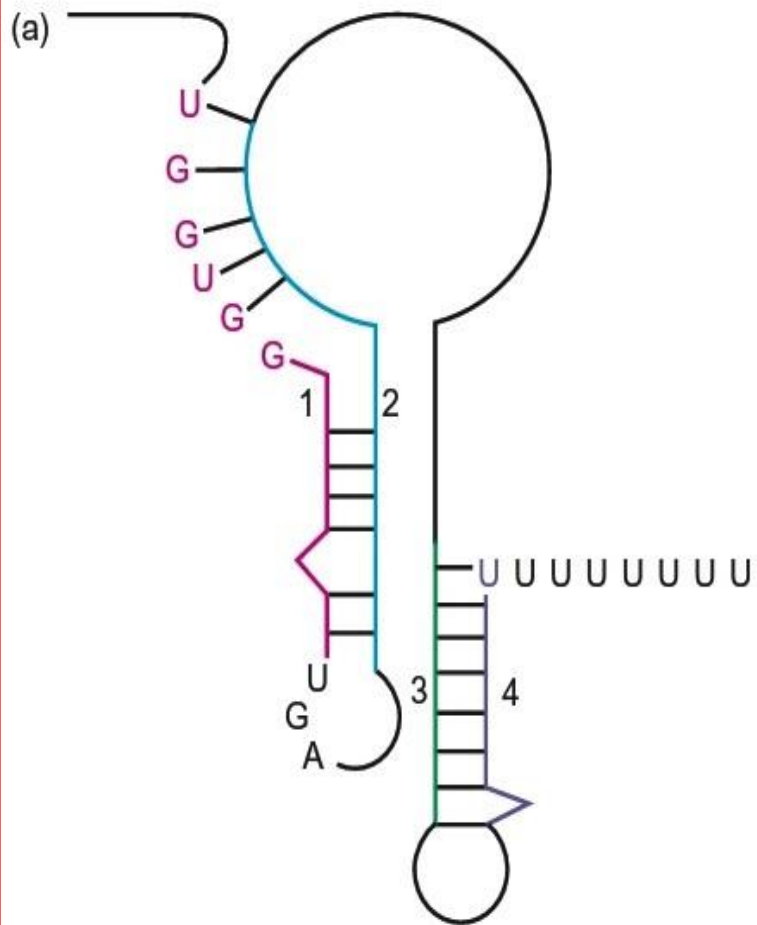


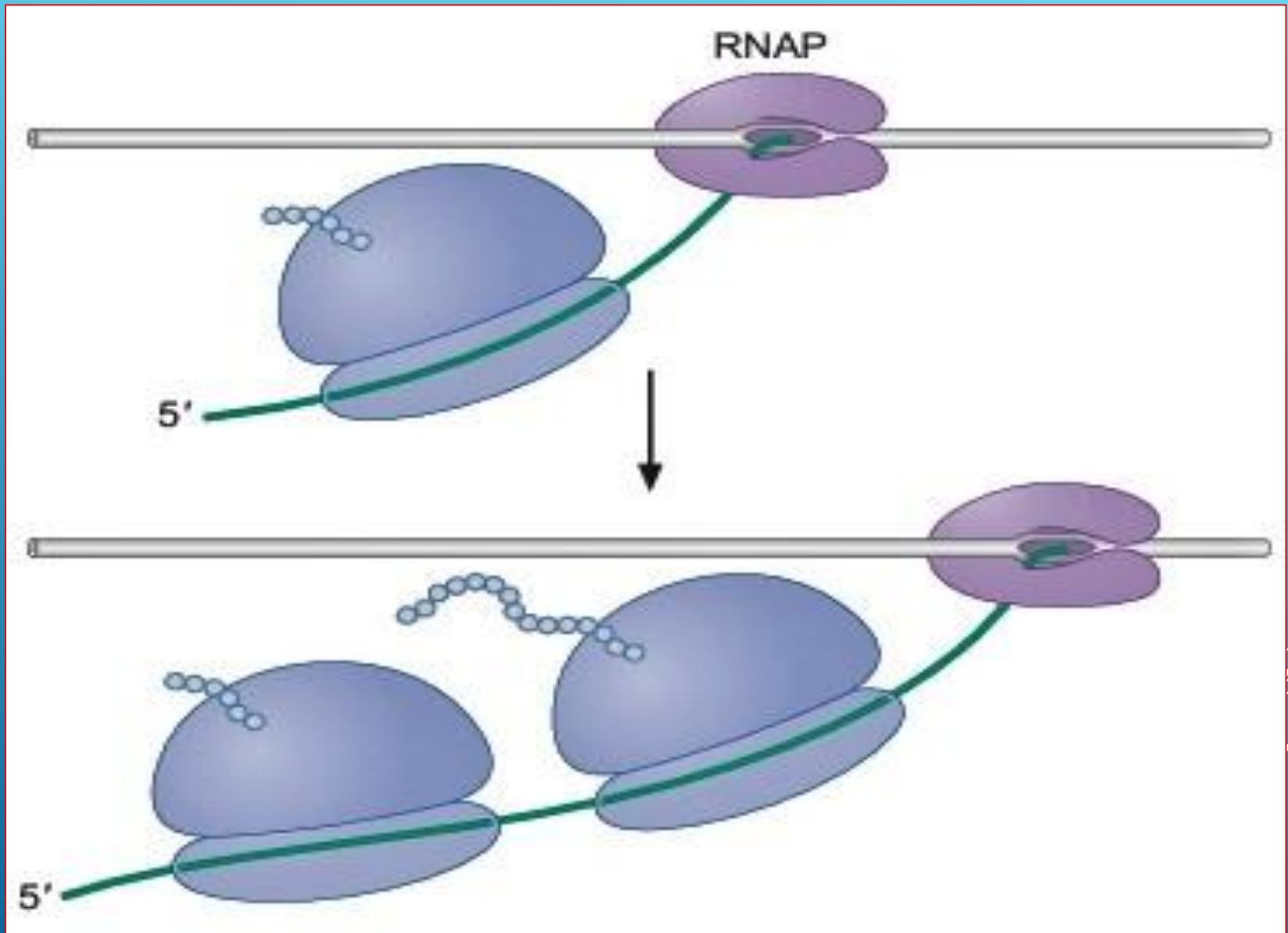
Regulation of trp operon by attenuation

The leader transcript itself forms stem-loop structures between region 1 and region 2 (1:2 hair-pin), between region 2 and region 3 (2:3 hair-pin) and between region 3 and region 4 (3:4 hair-pin). The 1st hair-pin acts as transcription pause signal, the 2nd hair-pin acts as anti-termination signal and the 3rd one acts as attenuation signal.

An essential feature of the attenuation in *E. coli* is the synchronization of translation of a 14-residue leader peptide coding region (*trpL*) with transcription of 162-nt leader region of the operon.







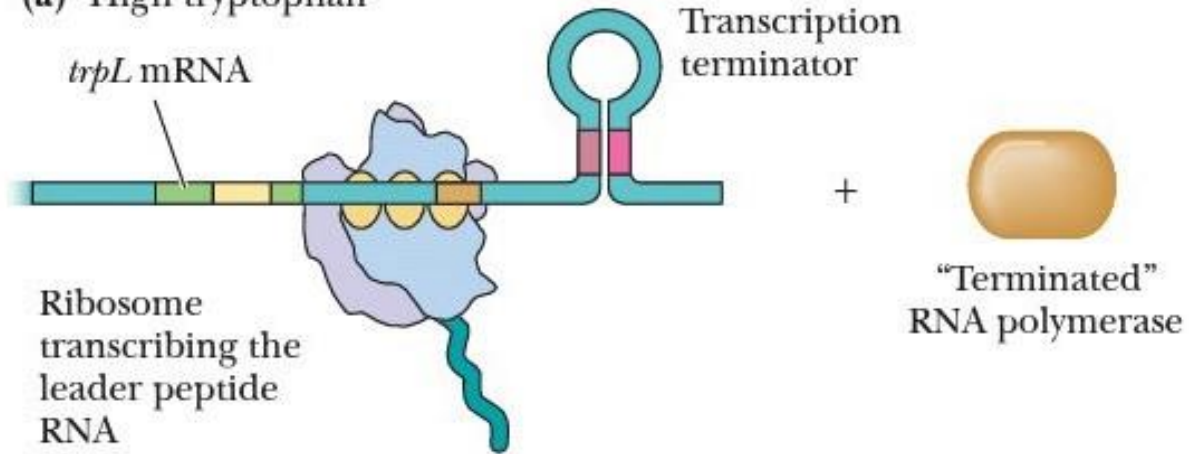
Regulation of trp operon at low level of tryptophan

When the level of tryptophan in a cell is low (tryptophan starvation condition), then the amount of charged tryptophenyl-tRNA^{trp} is also low. At this condition, the moving ribosome will be stalled at one of these two tandem Trp codons (UGGs) of region 1 of the leader transcript due to scarcity of charged tryptophenyl-tRNA^{trp}. This allows formation of antiterminator 2:3 hairpin that prevents formation of terminator 3:4 hairpin. As a result, transcription will continue into the structural genes of the operon, thus making tryptophan for a cell.

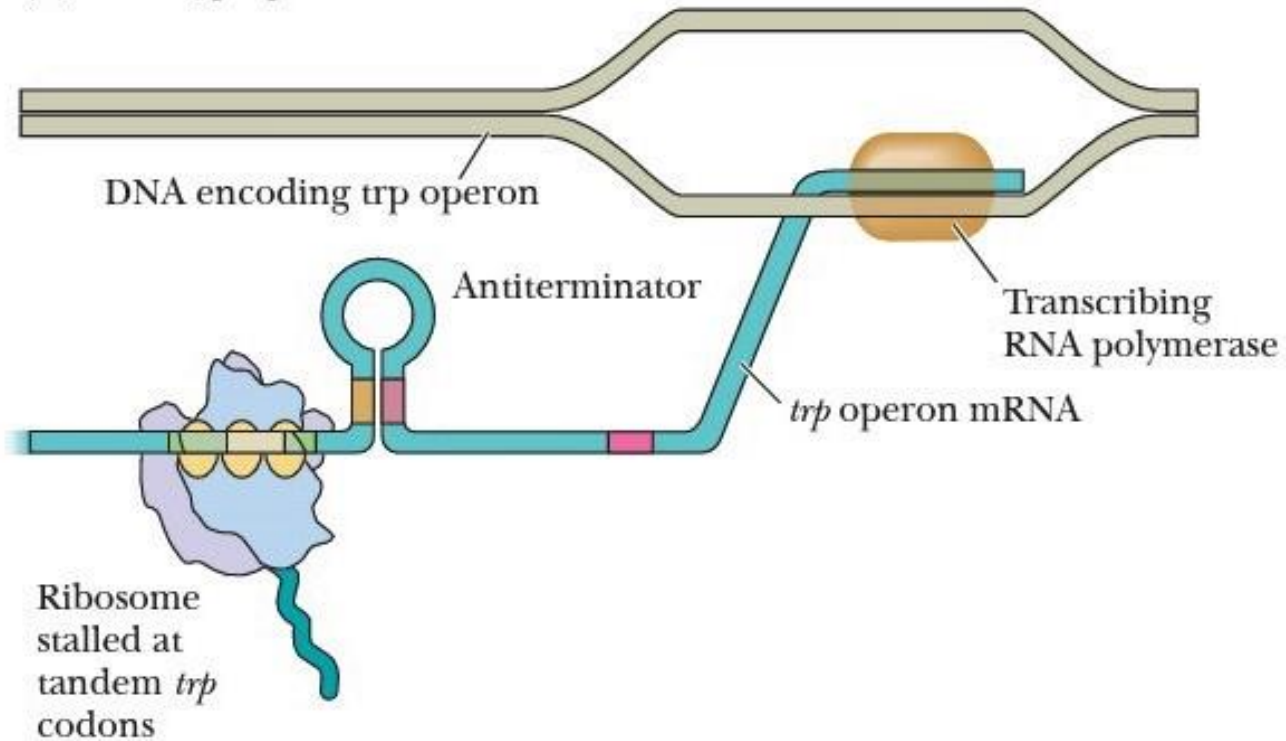
Regulation of trp operon at high level of tryptophan

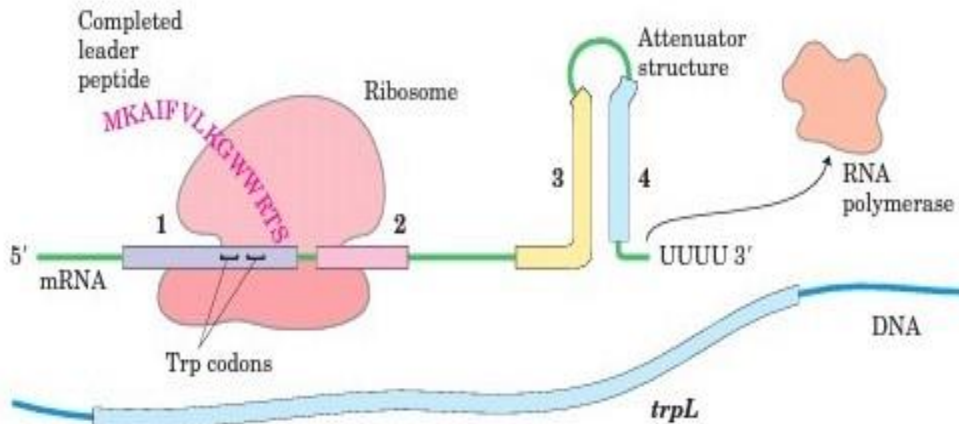
However, when the level of tryptophan in a cell is high (excess or abundant tryptophan condition), then the amount of charged tryptophenyl-tRNA^{trp} will also be high. Under this circumstance, the moving ribosome can continue the translation of two tandem Trp codons of the region 1 of the leader transcript but dissociates at stop codon (UGA). This permits region 1 to form a stem-loop structure with region 2 and so, the formation of the anti-antiterminator 1:2 hairpin allows formation of the terminator 3:4 hairpin. The moving ribosome may also pass two Trp codons of region 1 and enters into the region 2 before RNA polymerase synthesizing region 3 and region 4 of the leader mRNA. In this situation, as the region 2 is covered by the ribosome, it cannot pair with the region 3 when it (region 3) is synthesized, which allow formation of terminator 3:4 hairpin.

(a) High tryptophan

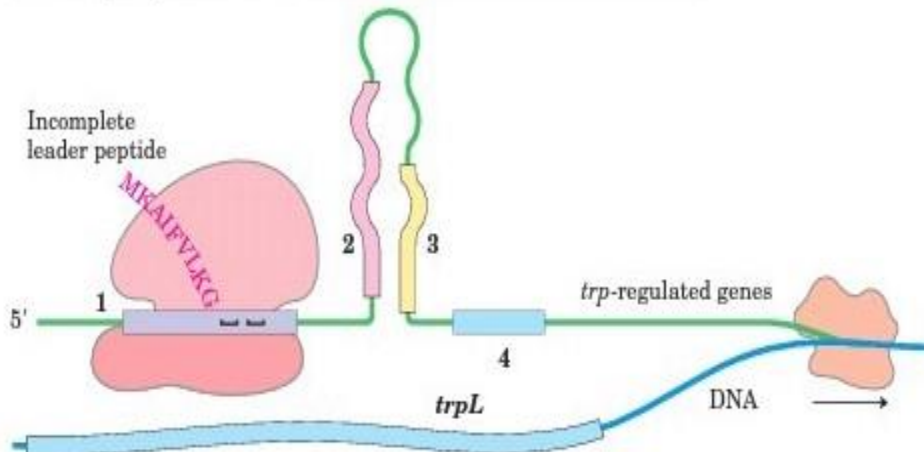


(b) Low tryptophan



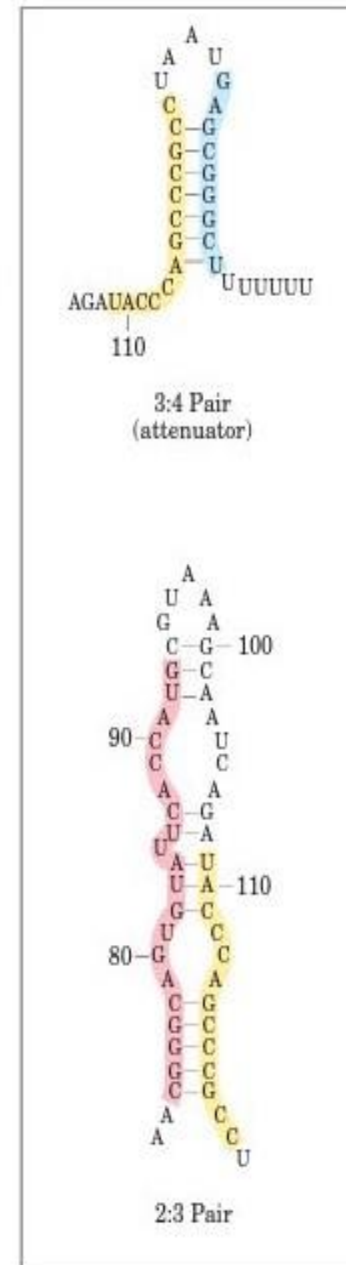


When tryptophan levels are high, the ribosome quickly translates sequence 1 (open reading frame encoding leader peptide) and blocks sequence 2 before sequence 3 is transcribed. Continued transcription leads to attenuation at the terminator-like attenuator structure formed by sequences 3 and 4.



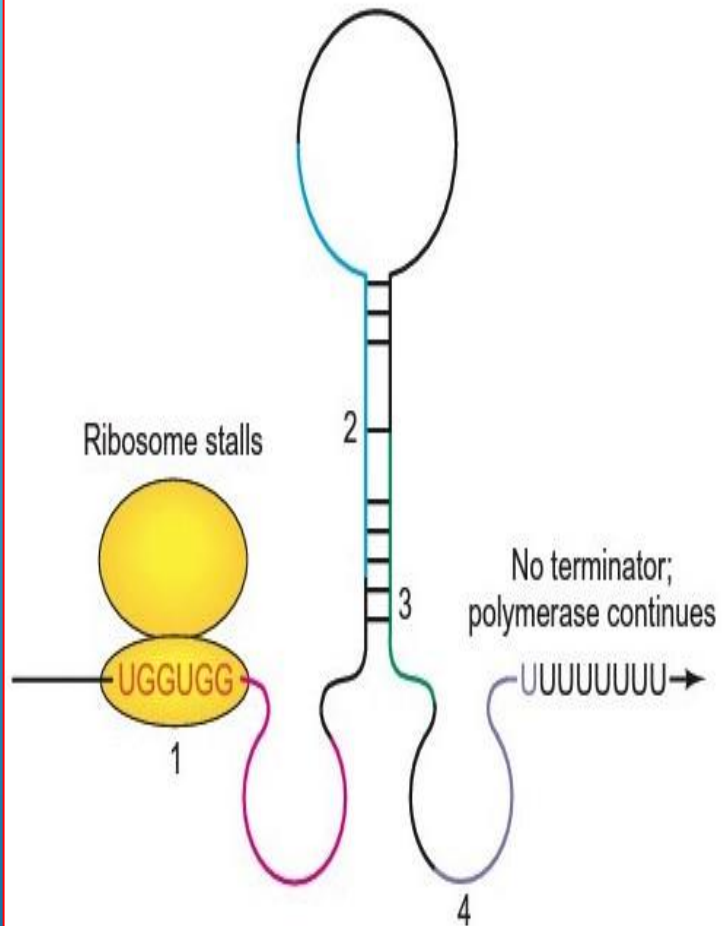
When tryptophan levels are low, the ribosome pauses at the Trp codons in sequence 1. Formation of the paired structure between sequences 2 and 3 prevents attenuation, because sequence 3 is no longer available to form the attenuator structure with sequence 4. The 2:3 structure, unlike the 3:4 attenuator, does not prevent transcription.

(b)



(c)

(a) Tryptophan starvation



(b) Tryptophan abundance

